Other support: U. S. Department of Health, Education and Welfare.

From the Thrombosis Research Institute, Department of Medicine, Temple University School of Medicine, Philadelphia.

BLOOD COAGULATION FACTOR XIa BINDS SPECIFICALLY TO A SITE ON ACTIVATED HUMAN PLATELETS DISTINCT FROM THAT FOR FACTOR XI

Binding of ^{125}I-Factor XIa to platelets required the presence of high molecular weight kininogen, was enhanced when platelets were stimulated with thrombin, and reached a plateau after 4-6 min of incubation at 37C. Factor XIa binding was specific: 50- to 100-fold molar excesses of unlabeled Factor XIa prevented binding, whereas Factor XI, prekallikrein, Factor XIIa, and prothrombin did not. When washed erythrocytes, added at concentrations calculated to provide an equivalent surface area to platelets, were incubated with Factor XIa, only a low level of nonspecific, nonsaturable binding was detected. Factor XIa binding to platelets was partially reversible and was saturable at concentrations of added Factor XIa of 0.2-0.4 μg/ml (1.25-2.5 μM). The number of Factor XIa binding sites on activated platelets was estimated to be 225 per platelet (range 110-450). We conclude that specific, high affinity, saturable binding sites for Factor XIa are present on activated platelets, are distinct from those previously demonstrated for Factor XI, and require the presence of high molecular weight kininogen.

Sinha, D., Seaman, F. ., Koshy, A., Knight, L. C., and *Walsh, P. N.*

Journal of Clinical Investigation 73:1550-1556, 1984.

Other support: National Institutes of Health and American Heart Association, Pennsylvania Affiliate.

From the Thrombosis Research Center, Section of Hematology/Oncology, Department of Medicine, Temple University School of Medicine, Philadelphia.

FACTOR V: A PLATELET CYTOSKELETAL ASSOCIATED PROTEIN

Platelet cytoskeletons prepared from thrombin activated platelets contain specifically associated Factor Va. We postulate that Factor Va associates with the cytoskeleton through receptors present on the platelet surface. The following evidence supports this hypothesis: (a) Prior secretion of Factor Va is necessary for the association of Factor Va on the cytoskeleton. (b) Reagents that inactivate Factor Va on the surface of the platelet, such as EDTA and proteolytic enzymes, also inactivate the Factor Va on the cytoskeleton. (c) The platelet Factor Xa binding site (Factor Va) are quantitatively retained on the platelet cytoskeleton. (d) Platelet cytoskeletons prepared from platelets that are thought to be deficient in Factor Va binding sites contain 25% of Factor Va activity of normal platelet cytoskeletons and platelet cytoskeletons prepared from platelets deficient in Factor Va contain no Factor Va activity but regain control levels of Factor Va only when Factor Va is added to the platelets prior to the preparation of cytoskeletons.

Tuszynski, G. P. and *Walsh, P. N.*

Haematologia 17(1):67-76, 1984.

Other support: U. S. Department of Health and Human Services.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

THE PLATELET CYTOSKELETON CONTAINS ELEMENTS OF THE PROTHROMBINASE COMPLEX

Triton-insoluble cytoskeletons prepared from thrombin-activated platelets were found to potentiate the activation of prothrombin (prothrombinase activity). Cytoskeletons prepared from red cells or lymphoblasts contained no prothrombinase activity. The platelet prothrombinase activity was dependent on cytoskeletal-associated Factor Va, and exogenously added Factor Xa and prothrombin. Cytoskeletons contained 38% of the total platelet prothrombinase activity. Both platelets and cytoskeletons displayed half-maximal activities at similar prothrombin concentrations. The role of lipids in the cytoskeletal prothrombinase activity was investigated. Cytoskeletons were found to contain 3.8% of the total platelet phospholipids, consisting of the following lipids expressed as percentage of total present in platelets: 6.0% sphingomyelin, 3.8% phosphatidylcholine, 2.9% phosphatidyl-ethanolamine, 4.4% phosphatidylinositol, and 2.2% phosphatidylserine. The cytoskeletal prothrombinase activity and the lipid phosphorus content of cytoskeletons decreased after treatment of cytoskeletons with various doses of phospholipase C. Incubation of cytoskeletons with the highest concentrations tested (10 μg/ml) resulted in a 72% loss of phosphatidylserine and 84% loss of cytoskeletal prothrombinase activity. Cytoskeletal prothrombinase activity destroyed by phospholipase C treatment could be restored to control levels by treatment of hydrolyzed cytoskeletons with total cytoskeletal lipid or mixtures of phosphatidylserine/phosphatidylcholine (25:75% by weight). These results suggest that the cytoskeletal prothrombinase complex in addition to containing Factor Va, as has been previously shown, contains a lipid cofactor activity consisting in part of phosphatidylserine.

Tuszynski, G. P., Maucos, G. P., Koshy, A., Schick, P. K., and *Walsh, P. N.*

The Journal of Biological Chemistry 259(11):6947-6951, 1984.

Other support: U. S. Department of Health and Human Services.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

COMPARISON OF BLEEDING TENDENCY, FACTOR XI COAGULANT ACTIVITY, AND FACTOR XI ANTIGEN IN 25 FACTOR XI-DEFICIENT KINDREDS

The relationship of clinical bleeding tendency and factor XI antigen (XI:Ag) in factor XI deficiency was studied in 78 members of 25 factor XI-deficient kindreds. Factor XI:Ag was measured in a competitive radioimmunoassay using monospecific, heterologous anti-factor XI antibody, ^{125}I-labeled factor XI, and staphylococcal protein A as the precipitating agent. Deficiency of factor XI clotting activity (XI:C), < 0.62 U/ml occurred in 48 individuals, 22 of whom experienced postoperative or posttraumatic bleeding. Their mean factor XI:C was 0.21 ± 0.04 U/ml (SEM) and factor XI:Ag was

0.23 ± 0.04 U/ml. The remaining 26 had no clinical bleeding, many despite surgical challenge. Their mean factor XI:C was 0.30 ± 0.04 U/ml and factor XI:Ag was 0.34 ± 0.05 U/ml. In all, 13 kindreds had between 1 and 11 members with bleeding; the other 12 had none with deficient hemostasis. Two heterozygous factor XI-deficient individuals appeared to be positive for cross-reacting material (CRM). The slope of the regression line for factor XI:C and factor XI:Ag data points in the 78 individuals tested did not differ from control and all points fell within 95% confidence limits derived from control. In conclusion, bleeding tendency appears to be consistent within a given kindred and is not determined exclusively by factor XI:C or factor XI:Ag levels.

Ragni, M. V., Sinha, D., Seaman, F., Lewis, J. H., Spero, J. A. and *Walsh, P. N.*

Blood 65(3):719-724, 1985.

Other support: National Institutes of Health.

From the Department of Medicine, University of Pittsburgh; Central Blood Bank, Pittsburgh; Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

FUNCTIONAL CHARACTERIZATION OF HUMAN BLOOD COAGULATION FACTOR XIa USING HYBRIDOMA ANTIBODIES.

During the initiation of intrinsic coagulation, factors XI and XIa interact intimately with several other coagulation proteins (factor XIIa, high M, kininogen, and factor IX) as well as with the platelet surface. To help elucidate these complex intramolecular interactions, we have prepared a collection of monoclonal antibodies directed against various epitopes in Factor XI. We have utilized these reagents to isolate factor XI and the light chain of factor XIa on affinity columns and to probe structure-function relationships involved in the interactions of factor XIa with factor IX. The isolated light chain of factor XIa retained > 90% of its amidolytic activity against the oligopeptide substrate pyro-Glu-Pro-Arg-pNA (S-2366), but only 3.8% of its clotting activity in a factor XIa assay and 1% of its factor IX activating activity in an activation peptide release assay. This suggests that regions of the heavy chain are required for development of coagulant activity and specifically for the interaction of factor XIa with factor IX. To test this hypothesis, the effects of three of the monoclonal antibodies (5F4, 1F1, and 3C1) on the function of factor XIa were examined. The results show that in a clotting assay the light chain-specific antibody (5F4) inhibits 100% of the factor XIa activity, whereas of the heavy chain-specific antibodies, one (3C1) inhibits 75% and another (1F1) only 17%. Similarly, in the factor IX activation peptide release assay antibody 5F4 inhibits 100% of the factor XIa activity, whereas 3C1 inhibits 75% and 1F1 inhibits 33%. We conclude that regions located in the heavy chain, in addition to those in the light chain, are involved in the interaction of factor XIa with factor IX and in the expression of the coagulant activity of factor XI.

Sinha, D., Koshy, A., Seaman F. S., and *Walsh, P. N.*

The Journal of Biological Chemistry 260(19):10714-10719, 1985.

Other support: U. S. Department of Health and Human Services, W.W. Smith Charitable Trust, American Heart Association Biomedical Research, and Division of Research Resources, National Institutes of Health.

From the Thrombosis Research Center, Department of Medicine, Temple University School of Medicine, Philadelphia, PA.

PLATELET-MEDIATED COAGULANT PROTEIN INTERACTIONS IN HEMOSTASIS

From the evidence reviewed here, it is possible to construct an overview of the hemostatic mechanism emphasizing the essential contributions of platelets in zymogen activations at each stage of intrinsic coagulation. Specific high-affinity receptors for various coagulation proteins are exposed on the platelet surface membrane. The coagulation proteins so far shown to bind the platelet membrane include the factor XI-high-mol wt kininogen complex, factor XIa, the factor Xa-factor Va complex, thrombin, and fibrinogen. As a consequence of these interactions, platelets promote the activation of prekallikrein, factor XII, factor XI, factor IX, factor X, and prothrombin. Platelets also appear to participate in a variety of alternative reaction mechanisms that may minimize or obviate the requirement for certain coagulation proteins, including factor XII and factor XI. This function of platelets may account for the absence of hemostatic defects in patients with deficiencies of some of the proteins involved in the contact phase of intrinsic coagulation. Finally, platelets appear to protect certain coagulation proteins, such as factor XIa and factor Xa, from inactivation by plasma proteinase inhibitors. These various functions of platelets are seen as localizing coagulation reactions to the hemostatic plug that occurs at loci of blood vessel injury.

Walsh, P. N.

Seminars in Hematology, 22(3): 176-186, 1985.

Other support: National Institutes of Health and American Heart Association.

From the Thrombosis Research Center, Temple University School of Medicine, Philadelphia.

POTENTIATION OF THE ACTIVITY OF COAGULATION FACTOR XI BY HUMAN PLATELETS

The present studies demonstrate that when washed platelets or platelet membranes are incubated with purified factor XI and the mixtures are assayed for factor XI activity, a 20-fold potentiation of coagulant activity is observed. This synergism is dependent on the presence of active factor XI and intact platelet membranes since it is abolished either by preincubation of factor XI with monospecific anti-factor XI antibody, or by preincubation of platelet membranes with phospholipase C. The saturable nature of the potentiation of purified factor XI by platelet membranes suggests the possibility that it represents a functional correlate of the saturable, specific binding of factor XI to platelets previously demonstrated. This suggestion is supported by the demonstration that platelets enhance the rate of proteolytic activation of factor XI in the presence of either factor XIIa or kallikrein.

Walsh, P. N. and Tuszynski, G. P.

Thrombosis Research 40:257-266, 1985.

Other support: National Institutes of Health, American Heart Association and W.W. Smith Charitable Trust.

From the Thrombosis Research Center, Department of Medicine, Temple University School of Medicine, Philadelphia.

EFFECT OF PROSTANOIDS ON THE CORONARY CIRCULATION

An accurate evaluation of the physiological and pathophysiological role of endogenous prostanoids in the human coronary circulation has to rely upon the joint results from studies using different techniques. In the present paper some data obtained in this laboratory are presented, which are aimed to elucidate the physiological and pathophysiological involvement of endogenous prostanoids in the regulation of coronary blood flow. Furthermore, some data on the pharmacology of PGI_2 in patients with acute myocardial infarction (AMI) are included. Based on the data presented here and on the considerations discussed it seems obvious that coronary prostanoid formation is limited both in healthy subjects and in patients with IHD. This is evidenced both by the lack of detectable release of PGI_2, the main coronary prostanoid formed, under normal conditions and under physiological stimulation of coronary blood flow, as well as under conditions of manifest cardiac ischaemia. On the other hand, the lack of significant release of PGI_2 is not due to a limited capacity to such formation, as evident from the marked release of 6-keto-$PGF_{1\alpha}$ in patients subjected to cardioplegia. Although the data presented here do not support the concept of a significant role for coronary prostanoids in health and disease the results presented on interaction between adenosine and prostanoids in the regulation of forearm blood flow may be of significance also in the coronary circulation, implying that prostanoids may play the role of a vasodilator messenger which transfers the signal to vascular relaxation from the endothelium to the vascular smooth muscle.

Wennmalm, A. et al.:

In: *IUPHAR 9th Int. Cong. of Pharmacol. London*, 1984. Proceedings, vol. 3, pp. 13-19, Macmillan Press, London, 1984.

Other support: The Swedish Medical Research Council and the National Research Council of Italy.

From the Department of Clinical Physiology, Karolinska Institute, Huddinge University Hospital, and Karolinska Hospital, Stockholm, Sweden; and Department of Pharmacology, Catholic University, Rome, Italy.

EFFECTS OF CHRONIC EXPOSURE TO CIGARETTE SMOKE ON AMINE LEVELS AND TURNOVER IN VARIOUS HYPOTHALAMIC CATECHOLAMINE NERVE TERMINAL SYSTEMS AND ON THE SECRETION OF PITUITARY HORMONES IN THE MALE RAT

Male rats were exposed to the smoke from 2 cigarettes every morning for a total period of 9 days. The next day they were decapitated immediately after the exposure to the smoke from 4 cigarettes (Kentucky reference 1R-1 type) burned at 30-min intervals. Control animals were exposed to air alone or to nicotine-free cigarette smoke (Cambridge glass fiber filters). In contrast to chronic exposure to filtered smoke, exposure to unfiltered smoke resulted in a 10% increase in catecholamine (CA) levels (quantitative histofluorimetry) within the lateral palisade zone, the posterior periventricular hypothalamic nucleus and within the dorsomedial hypothalamic nucleus. There was also an increase in amine turnover (tyrosine hydroxylase inhibition by α-methyl-*dl*-*p*-tyrosine methylester (αMT) in the dopamine (DA) systems of the medial and lateral palisade zones and in the periventricular noradrenaline (NA) hypothalamic systems. Chronic exposure to unfiltered cigarette smoke resulted in reductions of prolactin, LH and FSH levels (radioimmunoassay). Following αMT treatment, chronic exposure to unfiltered cigarette smoke still led to reduced prolactin serum levels. In addition, an increased vasopressin serum concentration was found. The effects of chronic exposure to cigarette smoke on neuroendocrine function and on hypothalamic CA systems are suggested to be mediated via nicotine. Combined with the results from a previous study, the present results indicate that tolerance does not develop with regard to the inhibitory effects of exposure to cigarette smoke on prolactin, LH and FSH secretions. The same is true for the stimulatory effects on the tubero-in-fundibular DA neurons and the periventricular NA systems. But chronic exposure to cigarette smoke seemed to induce tolerance with regard to its stimulatory effects on subependymal dorsomedial and paraventricular hypothalamic NA systems and on corticosteronesone release.

Andersson, K., Eneroth, P., *Fuxe, K.*, Mascagni, F., and Agnati, L. F.

Neuroendocrinology 41(6):462-466, 1985.

From the Department of Histology, Karolinska Institute, and Research and Development Laboratory, Department of Obstetrics and Gynecology, Karolinska Hospital, Stockholm; Department of Human Physiology and Endocrinology, University of Modena, Modena, Italy.

EFFECTS OF ACUTE CENTRAL AND PERIPHERAL ADMINISTRATION OF NICOTINE ON ASCENDING DOPAMINE PATHWAYS IN THE MALE RAT BRAIN. EVIDENCE FOR NICOTINE INDUCED INCREASES OF DOPAMINE TURNOVER IN VARIOUS TELENCEPHALIC DOPAMINE NERVE TERMINAL SYSTEMS.

The actions of intraventricular injections and intravenous infusions of nicotine were studied on dopamine stores and turnover in discrete areas of the forebrain of normal male rats. This was done by measuring the decline of the dopamine stores after tyrosine hydroxylase inhibition using α-methyl-tyrosine methyl ester (H 44/68). The dopamine concentrations in the various telencephalic dopamine nerve terminal systems were measured using the Falck-Hillarp methodology involving quantitative

microfluorimetry. The catecholamine concentrations in the anteromedial frontal cortex were measured biochemically, using high pressure liquid chromatography combined with electrochemical detection.

Intraventricular experiments. The dopamine levels in discrete areas of nuc. caudatus and nuc. accumbens were significantly reduced even with the lowest dose of nicotine (1 μg/rat). Intraventricular injections of nicotine in a dose of 100 μg/rat produced significant increases of dopamine turnover in various types of dopamine nerve terminal systems in the nuc. caudatus, nuc. accumbens, and tuberculum olfactorium, and following a dose of 10 μg/rat, increases of dopamine turnover were observed in the medial part of the nuc. caudatus. Furthermore, nicotine (100 μg/rat) significantly increased noradrenaline but not dopamine turnover with the anterofrontal cortex.

Intravenous experiments. The dopamine levels were selectively reduced by nicotine (1000 μg/kg) in the cholecystokinin positive and negative dopamine nerve terminal systems of the nuc. accumbens. On the other hand, dopamine levels in the anteromedial frontal cortex were increased after this dose of nicotine. Intravenous infusions of nicotine (10-1000 μg/kg) produced dose-related increases of dopamine turnover in the various dopamine nerve terminal systems analyzed in the telencephalon. These effects became significant with a dose of 1000 μg/kg/h. The dopamine terminals in the nuc. caudatus showed a higher sensitivity to intravenous infusions of nicotine, being affected by 10-100 μg/kg of nicotine.

These findings suggest that relatively low doses of nicotine via an activation of central nicotine-like cholinergic receptors can reduce dopamine concentration and increase dopamine turnover in discrete limbic and striatal areas. These actions may in part represent the neurochemical basis for the rewarding actions of nicotine and for nicotine dependence in man.

Andersson, K., *Fuxe, K.*, Agnati, L. F., and Eneroth, P.

Medical Biology 59:170-176, 1981.

Other support: Swedish Tobacco Company.

From the Department of Histology, Karolinska Institute, Stockholm, Sweden, and the University of Modena, Modena, Italy.

EFFECT OF MATERNAL NICOTINE ON THE DEVELOPMENT OF SITES FOR [^{3}H]NICOTINE BINDING IN THE FETAL BRAIN

The sites for [^{3}H]nicotine binding in fetal brains were examined after administration of nicotine into pregnant rats. Administration of unlabeled nicotine into the pregnant rats increased B_{max} values for the sites for tririared nicotine binding without affecting K_d values in the fetal brains. Treatment with this regimen, however, did not show any significant change in the sites for [^{3}H]quinuclidinyl benzylate binding. In addition, treatment with this regimen increased B_{max} values of the sites for [^{3}H]nicotine binding in the brains of pregnant rats. α-Bungarotoxin had no effect on the sites for [^{3}H]nicotine binding. It is inferred, therefore, that a similar response is elicited by nicotine binding sites to administered nicotine in both the fetal and maternal brains. Furthermore, a possible effect of nicotine in pregnant rats may be the facilitation of the development of nicotine acetylcholine receptors in the fetal brain.

Hagino, N. and Lee, J. W.

International Journal of Developmental Neuroscience 3(5):567-571, 1985.

From the Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio.

THE ROLE OF MATERNAL NICOTINE IN THE DEVELOPMENT OF NICOTINIC ACETYLCHOLINE RECEPTORS AND HYPOTHALAMIC DOPAMINERGIC NEURONS IN THE FETAL BRAIN

It has already been reported that delayed development of hypothalamic dopaminergic (TIDA) neurons in the fetal brain caused a delay of differentiation of prolactin cells and puberty onset in offspring of rats. From observations mentioned, it seems likely that maternal neurotransmitters and/or hormones could influence the development of TIDA neurons and differentiation of prolactin cells in offspring. Preliminary results indicate that maternal and placental acetylcholine (Ach) seem to act on nicotinic sites to activate the nicotinic acetylcholine receptors (nAchR) and influence the development of TIDA neurons in the fetal brain. This study tends to lend support to a working hypothesis that nAchRs appear in the fetal brain during an earlier stage of gestation. During the last week of gestation, maternal and placental Ach act on nicotinic sites to activate Ca^{2+} flux which stimulates TIDA neurons through activation of protein kinase and phosphorylation. Thus, maternal nicotine could influence the growth and development of TIDA neurons in the fetal brain.

Hagino, N.

In: Caciagli, F., Giacobini, E., and Paoletti, R. (eds.) *Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects*, New York: Elsevier Science Publishers B. V., 1984, pp. 127-130.

From the Department of Cellular and Structural Biology, The University of Texas Health Science Center, San Antonio.

[^{3}H]NICOTINE BINDING SITES IN DEVELOPING FETAL BRAINS IN RATS

[^{3}H]Nicotine binding sites were examined in developing fetal brains in rats. The fetal brain membranes bound [^{3}H]nicotine with an affinity similar to that of adult brain membranes. This binding was displaced by unlabeled nicotine or carbamylcholine, the inhibition concentrations being approximately the same for fetal and adult brain preparations. α-Bungarotoxin had no effect in [^{3}H]nicotine binding to fetal brains membranes as well as to adult brain preparations. The specific [^{3}H]nicotine binding was first detectable on day 16 of gestation and increased several fold until birth.

Sugiyama, H., *Hagino, N.*, Moore, G., and Lee, J. W.

Neuroscience Research 2:387-392, 1985.

From the Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, and the Department of Cellular Physiology, National Institute of Physiological Sciences, Okazaki, Japan.

GUANINE NUCLEOTIDES REGULATE [^{3}H]SUBSTANCE P BINDING IN RAT SMALL INTESTINE

The binding of [^{3}H]substance P (SP) to membranes of the rat small intestine demonstrates specific binding to receptors having more than one affinity for SP. The values of the binding parameters for the high-affinity site obtained from a non-linear

regression analysis are as follows: K_D = 0.25 nM, B_{max} = 149.5 fmol/mg protein. Inhibition curves of ^{3}H-SP binding using various unlabeled tachykinins show that the high-affinity receptor is of the P-subtype, having the highest affinity for SP and lower affinities for eledoisin and kassinin. Guanine nucleotides and sodium independently reduce the binding of ^{3}H-SP to the high-affinity receptor in a dose-related manner; GTP and GDP are more potent than GMP. The reduction of specific SP binding by GTP can be ascribed primarily to an increase in the off-rate. The effects of guanine nucleotides on ^{3}H-SP binding to membranes of rat small intestine suggest that the high-affinity receptor is linked to an effector by a GTP-binding regulatory protein.

Smith, K.E. and *Hoss, W.P.*

Regulatory Peptides 11:275-285, 1985.

Other support: National Institutes of Health.

From the Center for Brain Research, University of Rochester, School of Medicine and Dentistry, Rochester, NY.

CHARACTERIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS IN THE BRAINS OF COPPER-DEFICIENT RATS

In order to assess a possible role for copper as a regulator of muscarinic receptors *in vitro*, the receptor was characterized in rats made copper deficient by a dietary regimen. In forebrain regions there was a decrease in both the affinity of the receptors for [^{3}H]-1-quinuclidinyl benzilate and the density of receptors in the copper-deficient animals compared with control animals. Copper treatment *in vitro* of homogenates from deficient animals did not reverse the *in vivo* effects on antagonist binding but, rather, decreased receptor occupancy and ligand affinity in a manner similar to copper treatment of control homogenates. Minimally deficient rats displayed very similar changes in receptor properties compared with the more severely deficient animals. Minimal copper deficiency produced robust effects on the binding of agonists, increasing ID_{50} and derived dissociation constants. The addition of copper to the assay medium caused an apparent reversal of the *in vivo* effect of copper deficiency on agonist binding, decreasing ID_{50} and derived dissociation constants to values near those observed with homogenates from normal animals in the presence of copper. Since copper deficiency has dramatic effects on both receptor number and the binding of agonists to muscarinic receptors in the central nervous system, it is suggested that copper, because of its ability to form complexes with some proteins, may have an endogenous role in the regulation of the receptor.

Farrar, J. R., *Hoss, W.*, Herndon, R. M., and Kuzmiak, M.

The Journal of Neuroscience 5(4):1083-1089, 1985.

Other support: National Institutes of Health.

From the Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, NY.

PROTEIN PHOSPHORYLATION AND NEURONAL FUNCTION

Studies in the past several years have provided direct evidence that protein phosphorylation is involved in the regulation of neuronal function. Electro-

physiological experiments have demonstrated that three distinct classes of protein kinases, *i.e.*, cyclic AMP-dependent protein kinase, protein kinase C, and CaM kinase II, modulate physiological processes in neurons. Cyclic AMP-dependent protein kinase and kinase C have been shown to modify potassium and calcium channels, and CaM kinase II has been shown to enhance neurotransmitter release. A large number of substrates for these protein kinases have been found in neurons. In some cases (*e.g.*, tyrosine hydroxylase, acetylcholine receptor, sodium channel) these proteins have a known function, whereas most of them (*e.g.*, synapsin I) had no known function when they were first identified as phosphoproteins. In the case of synapsin I, evidence now suggests that it regulates neurotransmitter release. These studies of synapsin I suggest that the characterization of previously unknown neuronal phosphoproteins will lead to the elucidation of previously unknown regulatory processes in neurons.

Browning, M. D., *Huganir, R.*, and Greengard, P.

Journal of Neurochemistry 45(1):11-23, 1985.

Other support: National Science Foundation, U. S. Public Health Service, and a contract from the U. S. Air Force School of Aerospace Medicine.

From the Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University, New York.

EFFECT OF NICOTINE ON CEREBRAL PROTEIN METABOLISM DURING DEVELOPMENT

Several studies on the effects of nicotine on fetal growth have suggested that altered protein synthesis is the underlying mechanism of changes in gestational length, brain weight, or behavior. Discrepancies were found in gross structural changes; in some instances fetal size and brain weights were not found to be different after exposure to nicotine. However, most results indicate behavioral changes in the offspring. The results presented here suggest that nicotine does affect cerebral protein metabolism in both developing and adult brain. These researchers examined the rates of protein metabolism in the offspring of rats administered nicotine during gestation to assess whether changes in weight or protein content occur and whether they are related to alterations of protein metabolism. Results showed that there were no gross morphological changes in the offspring of rats administered nicotine during gestation. Cortical and cerebellar brain weights were similar to control values at 2 and 4 days after birth. Total protein content was also similar. However, measured synthesis rates were 20% and 5% lower in cortex of nicotine-treated animals at 2 and 4 days after birth, respectively. Calculated degradation rates (percent synthesis per h minus percent protein increase per h) are therefore lower in the nicotine animals to account for the lack of change in protein increase in these animals. Therefore, on the basis of this study, it appears that nicotine influences brain function, and that protein degradation and synthesis processes in fetal-newborn rat brain are affected by nicotine.

Sershen, H., Reith, M. E. A., and *Lajtha, A.*

In: Caciagli, F., Giacobini, E., and Paoletti, R. (eds.) *Developmental Neuroscience: Physiological, Pharmacological and Clinical Aspects*, New York: Elsevier Science Publishers B. V., 1984, pp. 119-122.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

NICOTINE BINDING SITES IN BRAIN

In the past 10 years, many attempts have been made to identify sites in brain that bind labeled nicotine. However, in these studies no estimation was made of saturable versus non-saturable binding. More recent studies show curvilinear Scatchard plots, suggesting the presence of multiple sites. Nicotinic or muscarinic antagonists have little or no affinity to the binding site, which has been suggested by Romano and Goldstein to be due to an agonist-induced shift of the receptor to a high-affinity agonist selective state. The kinetics of binding indicate a high-affinity component with a K_d in the range of 0.1-60 nM, and a low-affinity component with affinities that vary widely from one preparation to another. The present investigators have compared the binding of (±)-[^{3}H] nicotine and [^{3}H] acetylcholine in various brain regions Although midbrain showed the highest and cerebellum the lowest binding for both nicotine and acetylcholine, the ratio of nicotine/acetylcholine binding showed a three-fold regional variation. Although there is still much variability in the results of binding studies from various laboratories, nicotine binding in brain is probably not solely related to the classical nicotine receptors. Multiple binding sites have been reported, and may be related to the multiple effects of nicotine on the central nervous system.

Sershen, H., Reith, M. E. A., and *Lajtha, A.*

In: Caciaglia, F., Giacobini, E., and Paoletti, R. (eds.) Developmental Neuroscience: Physiogical, Pharmacological and Clinical Aspects, New York: Elsevier Science Publishers B.V., 1984, pp. 147-150.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

FREE RADICAL MEDIATED ALTERATIONS OF THE SYNAPSE

Previous work from this laboratory demonstrated that in the frog cutaneous pectoris neuromuscular junction, synaptic vesicles can be induced to reversibly fuse with the plasma membrane of the nerve terminal by the application of a Ringer's solution containing 105 mM K^+-propionate and that the exposure of this externalized vesicular membrane to horseradish peroxide (HRP) leads to vesicular membrane alterations during recycling. Similar HRP exposure of the synaptic vesicle membrane of 0.1 mm cerebral cortex slices results in both the reduction of high-affinity [^{14}C]-gamma-aminobutyric acid (GABA) uptake and in easily distinguishable vesicular membrane alterations after recycling. Brain slices initially incubated for 60 minutes in depolarizing (K^+) buffer containing HRP, followed by a 60-minute incubation in HEPES (synaptic vesicle recovery), exhibit a 30% reduction of [^{14}C]GABA uptake and the appearance of nonsynaptic vesicle membrane in the nerve terminal. The combination of these morphological and biochemical data points to the existence of a relationship between high-affinity neurotransmitter uptake and synaptic vesicle membrane. The fact that non-K^+-stimulated cerebral cortex slices (no synaptic vesicle-plasma membrane fusion) which are exposed to HRP (in HEPES) for 60 minutes exhibit no reduction of [^{14}C]GABA uptake indicates that fused vesicle membrane is susceptible to HRP damage and that damage to these membranes alters the neurotransmitter transport system. In order to isolate and better study the functional synaptic unit, synaptosomes (cerebral cortex) were prepared and exposed to HRP. In the absence of K^+-depolarization, synaptosomes exposed to HRP for 45 minutes take up 70% less [^{14}C]GABA. Observation of this HRP-induced reduction of synaptosomal neurotransmitter uptake in relation to the

HRP-induced synaptic vesicle membrane alterations observed in mouse cerebral cortex slices indicates that the plasma membranes of these isolated synaptosomal units are themselves susceptible to HRP-induced damage and that the damage of this membrane also alters neurotransmitter uptake.

Debler, E. A., Sershen, H., *Lajtha, A.*, and Gennaro, J. F., Jr.

Annals of the New York Academy of Sciences 435:140-144, 1984.

From the Department of Biology, New York University, and the Center for Neurochemistry, Ward's Island, New York.

ENDOGENOUS MATERIAL IN BRAIN INHIBITING [^{3}H]NICOTINE AND [^{3}H]ACETYLCHOLINE BINDING

The supernatant obtained from mouse brain homogenates contains material that inhibits the saturable binding of [^{3}H]nicotine in mouse cerebral cortex. This inhibitory material was further purified by heat denaturation, ultrafiltration through an Amicon PM-10 membrane filter, and gel chromatography on Sephadex G-10. The material inhibited the binding of [^{3}H]acetylcholine with the same potency as it did that of [^{3}H]nicotine. It also had some affinity for the sites that specifically bind [^{3}H]D-Ala, D-Leu enkephalin, but had much lower affinity for the binding sites for tritiated quinuclidinyl benzilate (QNB), spiroperidol, naloxone, or imipramine. Acid hydrolysis destroyed the activity. These preliminary results suggest the presence in brain of "nicotine-like" substances, one of which may be the endogenous ligand for the sites that specifically bind [^{3}H]nicotine.

Sershen, H., Reith, M. E. A., Hashim, A., and *Lajtha, A.*

Journal of Neuroscience Research 12(4):563-569, 1984.

Other support: New York State Health Research Council.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

COMPARISON OF [^{3}H]NICOTINE AND [^{3}H]ACETYLCHOLINE BINDING IN MOUSE BRAIN: REGIONAL DISTRIBUTION

In a continuing study of nicotine binding sites, the authors determined the relative amount of nicotine binding and acetylcholine binding in various brain regions of C57/BL and of DBA mice. Although midbrain showed the highest and cerebellum the lowest binding for both [^{3}H]nicotine and [^{3}H]acetylcholine, the ratio of nicotine to acetylcholine binding showed a three-fold regional variation. Acetylcholine inhibition of [^{3}H]nicotine binding indicated that a portion of nicotine binding was not inhibited by acetylcholine. These results indicate important differences between the binding of (±)-[^{3}H]nicotine and that of [^{3}H]acetylcholine.

Sershen, H., Reith, M. E. A., Hashim, A., and *Lajtha, A.*

Research Communications in Chemical Pathology and Pharmacology 48(3):145-352, 1985.

From the Center for Neurochemistry, Nathan S. Kline Institute for Psychiatric Research, Ward's Island, New York.

LOCAL AND SYSTEMIC CAPSAICIN PRETREATMENT INHIBITS SNEEZING AND THE INCREASE IN NASAL VASCULAR PERMEABILITY INDUCED BY CERTAIN CHEMICAL IRRITANTS

1. The effects of local exposure to chemical irritants and mechanical stimulation on sneezing reflexes have been studied in normal and capsaicin-pretreated, conscious guinea-pigs. The influence of local and systemic capsaicin pretreatment on vascular permeability to plasma proteins and the cardiovascular effects of local application of capsaicin to the nasal mucosa have also been studied in anaesthetized animals.

2. Local application of capsaicin (threshold dose 3 μM), nicotine (threshold dose 300 μM) or formalin to the nasal mucosa induced reflex sneezing discharges. Systemic or local capsaicin pretreatment abolished or reduced the sneezing responses to capsaicin and formalin. The response to nicotine was also reduced following local pretreatment with capsaicin, while the response to systemic pretreatment with capsaicin was only slightly affected. The sneezing response to mechanical stimulation was not affected by capsaicin pretreatment.

3. Pretreatment with a local anaesthetic induced a similar dose-dependent inhibition of the sneezing responses to both capsaicin and nicotine.

4. Local application of disodium cromoglycate to the nasal mucosa reduced the sneezing response to capsaicin, but not that to nicotine.

5. Local pretreatment with the 3 mM and 30 mM capsaicin solution inhibited the increase in vascular permeability to plasma proteins in the nasal mucosa induced by i.v. capsaicin. Local pretreatment with capsaicin did not result in any reduction in the capsaicin-induced permeability in the ureter, suggesting that such treatment did not have any major systemic toxic effects.

However, a small, acute increase in respiratory insufflation pressure, indicating bronchoconstriction, was seen when the 30mM capsaicin solution was applied to the nasal mucosa. The application of capsaicin (3 mM and 30 mM) to the nasal mucosa resulted in an increase in arterial blood pressure and tachycardia due to reflex sympathetic activation.

6. Exposure of normal guinea-pigs to an atmosphere saturated with ether caused excited avoidance behaviour and intense nose wipings with the fore paws. This response was abolished by systemic pretreatment with capsaicin and reduced by local capsaicin pretreatment.

7. Local application of serotonin, histamine, leukotriene C_4, bradykinin, phenyldiguanide, substance P (SP) or [d-Arg, d-Pro, d-Trp, Leu]SP, and SP-antagonist, did not induce any sneezing. High concentrations of compound 48/80 caused a small sneezing response. Local pretreatment with the SP-antagonist (7×10^{-4}M) did not influence the sneezing responses to nicotine or capsaicin.

8. It is concluded that only substances that are known to activate sensory nerves induce sneezing. Furthermore, there seems to be at least two types of afferent nerves in the nasal mucosa which respond to specific chemical irritation. One type, capsaicin-sensitive nerves, which respond to capsaicin, formalin, ether and nicotine, while another type of afferent nerves involved in sneezing reflexes is largely resistant to capsaicin pretreatment and is activated by nicotine. Local application of capsaicin to the nasal mucosa may thus be a selective way of reducing nasal reactivity to certain chemical irritants without causing systemic degeneration of capsaicin-sensitive C-fiber afferents.

Lundblad, L., *Lundberg, J. M.*, and Anggard, A.

Archives of Pharmacology 326:254-261, 1984.

Other support: Swedish Medical Research Council; the Swedish Tobacco Company; the Astra Foundation; the Swedish Society of Medical Science; and the Funds of the Karolinska Institute.

From the Department of Oto-Rhino-Laryngology, Karolinska Hospital, and the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

EFFECTS OF NEUROPEPTIDE Y (NPY) ON MECHANICAL ACTIVITY AND NEUROTRANSMISSION IN THE HEART, VAS DEFERENS AND URINARY BLADDER OF THE GUINEA-PIG

The effects of preincubation for 10 min with synthetic porcine neuropeptide Y (NPY) on muscle tone and autonomic transmission in the guinea-pig right atrium, vas deferens, urinary bladder, portal vein and trachea were analysed in vitro. NPY induced a metoprolol-resistant, long-lasting, positive inotropic and cronotropic effect *per se* in the spontaneously beating right atrium. Furthermore, NPY caused a reversible inhibition of both the metoprolol and atropine-sensitive auricle responses to field stimulation (2 Hz or 4 Hz for 2 s) without affecting the response to exogenous noradrenaline (NA) or acetylcholine (ACh).

NPY did not induce any contraction of the vas deferens, but inhibited both the rapid twitch response and the sustained tonic contraction induced by field stimulation. The NPY-induced inhibition of the tonic contraction was more long-lasting than than of the twitch response. The tonic contraction was blocked by phentolamine and the twitch response by α-, β-methylene ATP tachyphylaxis. NPY did not inhibit the contractile effects of NA, ATP or α-, β-methylene ATP. NPY also induced a reversible reduction of the non-cholinergic, not-adrenergic contractile response to field stimulation of the urinary bladder. In the portal vein, NPY (up to $5x10^{-7}$M) did not inhibit the spontaneous motility or the phentolamine-sensitive contractile responses to field stimulation and NA. The atropine-sensitive contraction of the trachea or the non-adrenergic, non-cholinergic relaxation induced by field stimulation was not significantly influenced by NPY in doses up to $5x10^{-7}$M. In conclusion, the present data show that in the guinea-pig, NPY exerts positive chronotropic and inotropic effects on the right atrium of the heart. Furthermore, NPY may have presynaptic effects on adrenergic, cholinergic and non-adrenergic—non-cholinergic neurotransmission.

Lundberg, J. M., Hua, X.-Y., and Franco-Cereceda, A.

Acta Physiologica Scandinavica 121:325-332, 1984.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, Astra Foundation, and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

CAPSAICIN-INDUCED STIMULATION OF THE GUINEA-PIG ATRIUM: INVOLVEMENT OF A NOVEL SENSORY TRANSMITTER OR A DIRECT ACTION ON MYOCYTES?

1. The mechanism underlying the positive inotropic and chronotropic effects of capsaicin were investigated using the spontaneously beating guinea-pig atrium *in vitro*.

2. Capsaicin induced a long-lasting stimulatory effect (threshold dose 10^{-9}M). Tetrodotoxin, phentolamine, 6-OHDA, mepyramine plus cimetidine, methysergide-, indomethacin-, somatostatin- or morphine pretreatment and local treatment with capsaicin on the vagal nerves did not reduce the capsaicin response, while it was abolished up to 1 month after systemic capsaicin pretreatment.

3. The capsaicin response was subject to a rapid tachyphylaxis. During capsaicin tachyphylaxis, the positive inotropic and chronotropic effects of noradrenaline, serotonin and histamine were unchanged.

4. Various neuropeptides were investigated with regard to cardiac activity. Physalaemin, eledoisin and somatostatin had negative inotropic and chronotropic effects. Substance P, bombesin, kassinin, CCK-8 or PHI (up to 10^{-6} M of each) did not cause any detectable response on the guinea-pig auricle, while the substance P antagonist [D-Arg, D-Pro, D-Trp, Leu]SP induced a long-lasting stimulation of heart activity. VIP also stimulated the heart.

5. Various adenyl compounds were also tested. Adenosine, AMP, ADP, ATP and β-, γ-methylene ATP had negative chronotopic and inotropic effects, while α-, β-methylene ATP induced a stimulatory response. During α-, β-methylene ATP tachyphylaxis, the auricles still responded to capsaicin. The inhibitory effects of adenosine and ATP analogues were antagonized by theophylline and 8-p-sulfophenyl theophylline. Capsaicin induced a small release of labeled nucleotides from ^{3}(H)-adenine-prelabeled atria from control, but not from capsaicin-pretreated animals.

6. GTP, aspartate and kainic acid (up to 10^{-4}M) had no effect on the guinea-pig atrium, while glutamate had a negative inotropic action.

7. In conclusion, the present findings show a specific stimulatory action of capsaicin on heart function. This effect does not seem to be mediated via any classical transmitter, including substance P and ATP. The capsaicin response was abolished by capsaicin pretreatment, which is known to cause degeneration of chemosensitive nerves in the heart. This suggests that capsaicin may release other bioactive substances than substances P from sensory nerves. A direct action of capsaicin on cardiac myocytes cannot be excluded. A desensitization phenomenon would then also occur on possible receptive sites for capsaicin on the myocytes. Capsaicin pretreatment may thus induce very long-lasting, specific, functional changes in heart function.

Lundberg, J. M., Hua, Y., and Fredholm, B. B.

Archives of Pharmacology 325:176-182, 1984.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Hans och Loo Ostermans Foundation, the Astra Foundation, and the Karolinski Institute.

From the Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

COMPARATIVE IMMUNOHISTOCHEMICAL AND BIOCHEMICAL ANALYSIS OF PANCREATIC POLYPEPTIDE-LIKE PEPTIDES WITH SPECIAL REFERENCE TO PRESENCE OF NEUROPEPTIDE Y IN CENTRAL AND PERIPHERAL NEURONS

Antisera raised against porcine neuropeptide Y (NPY) and peptide YY (PYY) were characterized with regard to immunohistochemical staining, cross-reactivity to several pancreatic polypeptide (PP)-related peptides, and radioimmunoassayable tissue levels in the rat and pig. The NPY antiserum (102B) reacted with nerves in many areas of both the central and peripheral nervous systems, but it did not stain endocrine cells of the pancreas or intestine. No evidence for any cross-reactivity of the NPY antiserum with related peptides of the PP family, such as avian PP, bovine PP, PYY, γ_1-MSH, FMRF-amide, or avian PP (31-36), was obtained. The NPY antiserum was N-terminally directed, and regional levels of NPY as seen by radioimmunoassay paralleled well the occurrence of NPY-immunoreactive structures seen in the immunohistochemical study. High pressure liquid chromatography analysis revealed that the NPY-immunoreactive

material from cerebral cortex and vas deferens had elution profiles similar to those of standard porcine NPY. The PYY antiserum mainly stained endocrine cells in the pancreas and intestine as well as a small neuron system in the brainstem of the rat. Although this antiserum had a slight cross-reactivity to NPY in radioimmunoassay, the neuronal PYY staining was separate from that of NPY. High levels of PYY were found in the intestine, and levels above the threshold were also seen in the dorsal vagal complex of the rat. The other antisera investigated (raised against avian PP, bovine PP, γ-MSH, and FMRF-amide) caused neuronal staining that was abolished by preabsorption with NPY. This was also seen even if no detectable cross-reactivity with NPY was found in radioimmunoassay. These latter antisera also stained endocrine cells in the pancreas and intestine with complex cross-reactivity relationships, suggesting the presence of intestinal PP-like peptides in addition to PYY and NPY.

Lundberg, J. M., Terenius, L., Hokfelt, T. and Tatemoto, K.

The Journal of Neuroscience 4/9:2376-2386, 1984.

Other support: Karolinska Institute, Astra Foundation, National Institute of Neurological and Communicate Disorders and Stroke, and the U. S. National Institute of Mental Health.

From the Departments of Pharmacology, Histology and Biochemistry, Karolinska Institute, Stockholm, Sweden; and the Department of Pharmacology, University of Uppsala, Sweden.

CORELEASE OF VASOACTIVE INTESTINAL POLYPEPTIDE AND PEPTIDE HISTIDINE ISOLEUCINE IN RELATION TO ATROPINE-RESISTANT VASODILATION IN CAT SUBMANDIBULAR SALIVARY GLAND

Parasympathetic nerve stimulation of the submandibular salivary gland in the cat caused salivary secretion, vasodilation and a corelease of vasoactive intestinal polypeptide (VIP) and peptide histidine isoleucine (PHI) immunoreactivities (IR) into the venous effluent, as indicated by an increase in output. The ratio between the released VIP-IR and PHI-IR was close to 1:1. Gel-permeation chromatography of plasma from the submandibular venous effluent indicated that the released VIP-IR and PHI-IR were very similar to porcine VIP and PHI, respectively. Atropine pretreatment enhanced output of both VIP-IR and PHI-IR during the parasympathetic nerve stimulation to a similar extent (about 5-fold) compared to control stimulations. This increase could be due to an inhibitory presynaptic muscarinic receptor regulation of VIP and PHI release. Since VIP and PHI are present in the same postganglionic parasympathetic nerves in the gland and both peptides have vasodilator activity, the present data suggest that both VIP and PHI may contribute to the atropine-resistant vasodilation seen upon stimulation of the chorda-lingua nerve. The parasympathetic control of salivary gland function may thus involve a multimessenger system with the classical transmitter acetylcholine and the peptides VIP and PHI.

Lundberg, J. M., Fahrenkrug, J., Larsson, O., and Anggard, A.

Neuroscience Letters 52:37-42, 1984.

Other support: P. Carl Petersen's Fund, NOVO Foundation, Swedish Medical Research Council, Swedish Tobacco Company, Astra Foundation, Swedish Society for Medical Sciences, Karolinska Institute, and Swedish Patent Revenue Research Fund.

From the Department of Pharmacology, Karolinska Institute, Department of Oto-Rhino-Laryngology, Karolinska Hospital, Stockholm, Sweden; Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark.

COMPARISON OF CARDIOVASCULAR AND BRONCHOCONSTRICTOR EFFECTS OF SUBSTANCE P, SUBSTANCE K AND OTHER TACHYKININS

1. The effects of substance P (SP), substance K (SK), physalaemin, eledoisin, kassinin, neuromedin K and bombesin on blood pressure, heart rate, respiratory insufflation pressure and plasma extravasation were studied in the guinea-pig.

2. All tachykinins except neuromedin K caused a fall in blood pressure with rather similar potency. The hypotensive response after physalaemin was comparatively more long-lasting.

3. SK and eledoisin (2.5 nmol × kg^{-1} i.v.) caused an initial bradycardia which then changed into tachycardia. The other tachykinins induced a slowly developing tachycardia. Neuromedin K (up to 40 nmol × kg^{-1}) did not influence heart rate.

4. SK, kassinin and eledoisin were more potent than SP and physalaemin in increasing respiratory insufflation pressure. The effect of SK had a particularly long duration. Neuromedin K only induced a weak increase in insufflation pressure at a very high dose.

5. All tachykinins except neuromedin K induced an increase in vascular permeability to plasma proteins in many visceral organs, as indicated by Evans blue extravasation. The trachea and ureter were the most sensitive organs with regard to this effect. Physalaemin and eledoisin were generally more potent in increasing vascular permeability in various organs than SP and SK. The maximal permeability-increasing effect of SK was smaller than that of SP, although the potency was similar.

6. Bombesin increased insufflation pressure with no clearcut effect on vascular permeability.

7. It is concluded that in the same species, *i.e.*, guinea-pig, several tachykinins have rather similar hypotensive action, while the vascular permeability increase to plasma proteins is especially pronounced after physalaemin and eledoison. SK, kassinin and eledoisin have prominent bronchoconstrictor effects. Neuromedin K, however, displays poor activity in the present models. The existence of novel tachykinins such as SK in addition to SP in mammalian tissues suggests that effects seen upon antidromic stimulation of sensory nerves may be caused by several structurally related peptides.

Hua, X. -Y., *Lundberg, J. M.*, Theodorsson-Norheim, E., and Brodin, E.

Archives of Pharmacology 328:196-201, 1984.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Astra Foundation; and the Karolinska Institute.

From the Department of Pharmacology, Karolinska Institute, and the Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden.

CO-EXISTENCE OF PEPTIDE HI (PHI) AND VIP IN NERVES REGULATING BLOOD FLOW AND BRONCHIAL SMOOTH MUSCLE TONE IN VARIOUS MAMMALS INCLUDING MAN

By immunohistochemistry it was found that PHI- and VIP-like immunoreactivity (IR) occurred in the same autonomic neurons in the upper respiratory tract, tongue and

salivary glands with associated ganglia in rat, guinea pig, cat, pig and man. VIP- and PHI-like immunoreactivity were also found in similar locations in the human heart. The N-terminally directed, but not the C-terminally directed, PHI antiserum or the VIP antiserum stained endocrine cells in the pig duodenum. This suggests the existence of an additional PHI-like peptide. Ligation of nerves acutely caused marked overlapping axonal accumulations of PHI- and VIP-IR central to the lesion. Two weeks after transection of the nerves, both types of immunoreactivities were still observed in accumulations both in the axons as well as in the corresponding cell bodies. The levels of PHI- and VIP-IR in normal tissues from the cat were around 10-50 pmol/g with a molar ratio of about 1 to 2. Systemic administration of PHI and VIP induced hypotension, probably due to peripheral vasodilatation in both guinea pig and cat. Furthermore, both PHI and VIP caused an inhibition of the vagally induced increase in respiratory insufflation pressure in guinea pig. PHI and VIP relaxed the guinea pig trachea *in vitro*, suggesting a direct action on tracheobronchial smooth muscle. VIP was about 5-10 times more potent than PHI with regard to hypotensive effects and 2-3 fold, considering respiratory smooth muscle-relaxant effects in the guinea pig. PHI was about 50-fold less potent in inducing hypotension in the cat than in the guinea pig. Although species differences seem to exist as regards biological potency, PHI should also be considered when examining the role of VIP as an autonomic neurotransmitter.

Lundberg, J. M. et al.

Peptides 5:593-606, 1984.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, NOVO Foundation, Astra Foundation, and the Karolinska Institute.

RELEASE OF SUBSTANCE P- AND SUBSTANCE K-LIKE IMMUNOREACTIVITIES FROM THE ISOLATED PERFUSED GUINEA-PIG LUNG

In this study, the authors offer direct evidence for a release of SP- and SK-like immunoreactivities (LI) by chemical irritation from the isolated perfused guinea-pig lung. Chemical irritation of tissues was achieved by perfusion with buffer containing 1 μM capsaicin. SP-LI was measured with the antibody Rd 2 which does not cross-react to SK. SK-LI was measured with the antibody K12-8307 which does not show cross-reactivity to SP. Infusion of capsaicin induced a several-fold increase in the outflow of SP-LI and SK-LI. Capsaicin, a strong irritant of the respiratory tract, excites and, in high doses, selectively destroys sensory C-fibers. Furthermore, a calcium requiring release of SP from sensory neurons of the guinea-pig ureter by capsaicin has been previously demonstrated. Thus, the present results indicate that SK-LI in the respiratory tract is, like SP, contained in primary afferent C-fibers. Capsaicin-sensitive C-fibers are responsible for both bronchoconstriction and tracheobronchial edema after chemical irritation. SP has been postulated as one mediator of these responses mainly because of the inhibitory effects of SP antagonists which, however, inhibit also actions of other tachykinins.

Saria, A., Theodorsson-Norheim, E., Gamse, R., and *Lundberg, J. M.*
European Journal of Pharmacology 106:207-208, 1985.

Other support: Austrian Scientific Research Fund, Swedish Tobacco Company, Petrus and Augusta Hedlunds Foundation and the Swedish Medical Research Council.

From the Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria; Department of Clinical Chemistry, Karolinska Hospital and Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

DIFFERENTIAL EFFECTS OF RESERPINE AND 6-HYDROXYDOPAMINE ON NEUROPEPTIDE Y (NPY) AND NORADRENALINE IN PERIPHERAL NEURONS

1. The effects of 6-hydroxydopamine (6-OHDA) and reserpine pretreatment on peripheral neuropeptide Y (NPY)- and noradrenaline (NA)-containing neurons were studied in guinea-pigs.

2. Ten days after 6-OHDA pretreatment a 60-80% reduction of the NA content was observed in the right atrium of the heart, stellate ganglion and spleen. The content of NPY-like immunoreactivity (LI) was reduced by about 50% in the heart, and was unchanged in the spleen while it increased to 200% of control in the stellate ganglion. Immunohistochemistry showed a pronounced loss of NPY- and tyrosinehydroxylase (TH)-immunoreactive (IR) nerves in the heart but not in the spleen. Increased NPY-IR was seen in axons and cell bodies of the stellate ganglion.

3. Reserpine pretreatment (threshold dose 0.5 mg X kg^{-1}) caused a dose- and time-dependent reduction of the content of NPY-LI in the heart. A maximal depletion of NPY-LI (about 80%) was observed 5 days after reserpine. Reserpine pretreatment also reduced the content of NPY-LI in the spleen, while no significant change was observed in the adrenal gland or vas deferens. The levels of NPY-LI increased in the stellate ganglion to about 180% of control 5 days after reserpine. Immunohistochemical analysis revealed an almost total loss of NPY-IR nerve fibres in the heart as well as around blood vessels in the lung and skeletal muscle. No detectable changes were observed in perivascular NPY-IR nerves in the spleen, vas deferens or kidney. TH-IR nerves remained unchanged after reserpine, indicating that the observed loss of NPY-IR nerves was due to a depletion of NPY and not a degeneration.

4. No change in the levels of substance P-LI was observed in the right atrium 5 days after reserpine.

5. NA was, in contrast to NPY, markedly depleted in all tissues investigated after reserpine treatment. The depletion of NA was more extensive, and occurred more rapidly and at much lower doses as compared to the effects on NPY-LI.

6. Ligations of the sciatic nerve revealed that NPY-LI was transported axonally with a rapid rate (3 mm/h). Reserpine pretreatment significantly increased the amount of accumulated NPY-IR above the ligation, suggesting an increase in axonal transport.

7. High performance liquid chromatography revealed that the NPY-LI consisted of two major peaks in the stellate ganglia, while only one peak closely corresponding to porcine NPY was seen in the right atrium.

8. In conclusion, 6-OHDA pretreatment depletes NPY-LI in certain terminal regions and increases NPY-LI in ganglia. Reserpine induces a tissue- and dose-dependent depletion of NPY-LI in certain terminal areas, while corresponding cell body content and axonal transport of the peptide seem to increase.

Lundberg, J. M. et al.

Archives of Pharmacology 328:331-340, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Astra Foundation, Swedish Society for Medical Sciences, the Karolinska Institute, and Austrian Scientific Research Funds.

From the Departments of Pharmacology and Histology, Karolinska Institute, Stockholm, Sweden; Department of Experimental and Clinical Pharmacology, University of Graz, Graz, Austria; Department of Pharmacology, University of Uppsala, Sweden; and the Department of Psychiatry, New York University Medical Center, New York.

CAPSAICIN PRETREATMENT INHIBITS THE FLARE COMPONENT OF THE CUTANEOUS ALLERGIC REACTION IN MAN

In this study the authors have investigated whether capsaicin pretreatment could impair the cutaneous triple response reaction to antigen challenge in man. The skin of 6 volunteers (3 males, 3 females age 29-40) with an established allergy to rats was pretreated locally with capsaicin. Capsaicin pretreatment produced a burning sensation and flare reaction but no wheal. Injection of rat allergen into control skin area resulted in instant itching followed by a progressively developing flare and wheal reaction. Capsaicin pretreatment of the skin thus almost totally abolished the acute flare component and reduced the itching sensation of the cutaneous allergy reaction in man. This suggests that capsaicin-sensitive sensory nerve endings are activated by mediators released upon mast cell degranulation by allergen exposure. Nerve activation then probably results in the release of vasoactive peptides such as substance P which mediate the flare reaction. The wheal response, however, was unchanged after capsaicin pretreatment. This suggests that activation of sensory nerves plays a major role in the flare and itching reaction while the wheal response is apparently to a major extent independent of mediator release from capsaicin-sensitive nerves.

Lundblad, L., *Lundberg, J. M.*, Anggard, A., and Zetterstrom, O.

European Journal of Pharmacology 113:461-462, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, and Petrus and Augusta Hedlunds Foundation.

From the Departments of Oto-Rhino-Laryngology and Thoracic Medicine, Karolinska Hospital, and Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

SUBCELLULAR STORAGE AND AXONAL TRANSPORT OF NEUROPEPTIDE Y (NPY) IN RELATION TO CATECHOLAMINES IN THE CAT

The subcellular storage of neuropeptide Y-like immunoreactivity (NPY-LI) in peripheral sympathetic neurons and adrenal gland as well as its axonal transport in the sciatic nerve was studied in relation to catecholamines in the cat. In the subcellular fractions from different parts of sympathetic neurons, *i.e.*, cell bodies (coeliac ganglia), axons (sciatic nerve) and terminals (spleen), the NPY-LI was found together with noradrenaline (NA) in heavy fractions assumed to contain large dense-cored vesicles. In addition, minor lighter fractions in the coeliac ganglion contained NPY-LI. The molar ratio between vesicular NA and NPY was high in the terminal regions (150 to 1) and much lower in axons and cell bodies (10 to 1), thus reflecting the different mechanisms of resupply for classical transmitter and peptide. In the adrenal gland the NPY-LI was mainly located in the catecholamine-storing chromaffin-granule fraction and also to a smaller extent in lighter fractions. Using reversed-phase HPLC, one molecular form of NPY-LI corresponding to porcine NPY was found in the coeliac ganglion, while the adrenal medulla also contained minor peaks with NPY-LI in addition to the main form,

which co-eluted with porcine NPY. NA was stored both in light and heavy fractions in the spleen, while it was mainly found in heavier fractions in the sciatic nerve. In the coeliac ganglion, most of the noradrenaline was present in a non-particulate form. The anterograde transport rate for NPY-LI in the sciatic nerve was estimated to be about 9 mm h^{-1}. A minor retrograde transport of NPY-LI was also detected. In conclusion, the present data suggest that NPY, a peptide with sympathoactive actions, is co-stored with NA in heavy fractions corresponding to large dense-cored vesicles, while light fractions with small dense-cored vesicles probably contain NA but not NPY-LI. The main resupply of NPY to terminals is, in contrast to NA, most likely by axonal transport, which implicates differences in the storage, turnover and release of these co-existing substances in the sympathoadrenal system.

Fried, G., *Lundberg, J. M.*, and Theodorsson-Norheim, E.

Acta Physiologica Scandinavica 125:145-154, 1985.

Other support: Swedish Medical Research Council, Swedish Society for Medical Sciences, Swedish Tobacco Company, Hedlund's Foundation, and the Karolinska Institute.

From the Department of Physiology and Pharmacology, Karolinska Institute, and Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden.

VIP AND PHI IN CAT NEURONS: CO-LOCALIZATION BUT VARIABLE TISSUE CONTENT POSSIBLE DUE TO DIFFERENTIAL PROCESSING

The concentrations of vasoactive intestinal polypeptide (VIP) and the peptide with NH_2-terminal histidine and COOH-terminal isoleucine (PHI) in various peripheral tissues and some areas in the CNS of the cat were compared with their immunohistochemical localization. The VIP levels in the gastrointestinal tract were 3 to 6 times higher than PHI levels. Much (up to 10-fold) higher VIP than PHI levels were also observed in the genitourinary tract as well as in the lung and heart. In the neurohypophysis, however, the VIP/PHI ratio was close to 1. Gel-permeation chromatography revealed that VIP- and PHI-immunoreactivity (IR) in the intestine, pancreas and brain consisted of three larger molecular forms in addition to the "standard" peptides. These larger forms which had overlapping elution positions may represent prepro-VIP/PHI forms. The immunohistochemical analysis revealed that VIP- and PHI-IR were present in the same ganglion cells in the intestine, pancreas, uterus and sympathetic ganglia. Furthermore, the terminal networks for these two peptides were very similar in the periphery. In the median eminence of the hypothalamus and in the posterior lobe of the pituitary, considerably more nerves were PHI- than VIP-IR. This observation was in parallel to a low VIP/PHI ratio. In conclusion, VIP and PHI seem to co-exist in most neuronal systems. Although the ratio of VIP and PHI on the precursor gene is 1:1, differences in posttranslational processing may create a considerably higher content of VIP than PHI in [illegible] terminal areas.

Fahrenkrug, J., Bek, T., *Lundberg, J. M.*, and Hokfelt, T.

Regulatory Peptides 12:21-34, 1985.

Other support: NOVO Foundation, the P. Carl Petersen's Foundation, the Danish Hospital Foundation for Medical Research, the Swedish Council for Medical Research, the Swedish Tobacco Company, Petrus och Augusta Hedlunds Stiftelse, and the Karolinska Institute.

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EVIDENCE FOR SPECIFIC NEUROPEPTIDE Y-BINDING IN RAT BRAIN SYNAPTOSOMES

Neuropeptide Y (NYPY), a neuropeptide with N- and C-terminal tyrosine (Y), is one of the major peptides in the brain and peripheral nervous system. NPY co-exists with noradrenaline (NA) in neurons in both the CNS and periphery but is also present in non-catecholaminergic systems. Sympathetic activation induces co-release of NPY and NA, subsequently these two agents cooperate in functional responses such as vasoconstriction. NPY has therefore been proposed to be a co-transmitter together with NA. In the present study, further evidence is provided for NPY being a neurotransmitter since specific binding sites for ^{125}I-NPY exist in brain membranes of the rat. As demonstrated in this study, the affinity of ^{125}I-NPY to rat brain synptosomes and the number of binding sites are of a magnitude similar to that for receptors described for other putative peptidergic transmitters. ^{125}I-NPY binding could be displaced by unlabeled NPY and PYY, but not by other structurally related peptides from the pancreatic polypeptide family or neurotensin. Therefore, the investigated binding sites show a high specificity for NPY and PYY. However, NPY is probably the endogenous ligand for these binding sites, since the K_i value for NPY was higher than that for PYY and, moreover, PYY does not seem to be present in most areas of the rat brain. The demonstration of specific, high affinity binding sites for ^{125}I-NPY, which have characteristics of pharmacological receptors, further supports the proposal that NPY is an important factor in neurotransmission.

Saria, A., Theodorsson-Norheim, E., and *Lundberg, J. M.*

European Journal of Pharmacology 107:105-107, 1985.

Other supports: Swedish Medical Research Council, Swedish Tobacco Company and Petrus and Augusta Hedlunds Foundation.

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NEUROPEPTIDE Y (NPY): ENHANCEMENT OF BLOOD PRESSURE INCREASE UPON α-ADRENOCEPTOR ACTIVATION AND DIRECT PRESSOR EFFECTS IN PITHED RATS

The effects of neuropeptide Y (NPY) on blood pressure and heart rate were studied in pithed rats. System infusion of NPY in a dose (230 pmol X kg^{-1} X min^{-1}) which *per se* did not affect blood pressure enhanced the pressor response to phenylep... X kg^{-1} i.v.) and that to electrical stimulation of the sympathetic outflow. In higher doses, NPY caused a pressor effect per sen, which was dose-dependently antagonized by nifedipine but not by adrenocaptor antagonists. In conclusion, NPY enhanced the α-adrenoceptor-mediated response and had Ca^{2+}-dependent vasoconstrictor activity *in vivo*.

Dahlof, C., Dahlof, P., and *Lundberg, J. M.*

European Journal of Pharmacology 109:289-292, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Karolinska Institute, the Swedish Society for Medical Sciences, and the Astra Foundation.

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NEUROPEPTIDE Y AND SYMPATHETIC CONTROL OF HEART CONTRACTILITY AND CORONARY VASCULAR TONE

The effects of neuropeptide Y (NPY) on contractility of the spontaneously beating guinea-pig atrium and transmural nerve stimulation (TNS)-induced efflux of tritium-noradrenaline (^{3}H-NA) were studied *in vitro*. NPY induced a moderate positive chronotropic and inotropic atrial response which was resistant to metroprolol. TNA at 2 Hz for 2 s caused an increase in rate and contractile force. These effects were significantly reduced by NPY. NYP also reduced the TNS-induced (2 Hz for 20 s), fractional [^{3}H]NA release by 40% without affecting the contractile response. The contractile effects of exogenous NA on the guinea-pig atrium were not affected by NPY. NPY caused a long-lasting increase in coronary perfusion pressure and also, in high doses, an inhibition of ventricular contractility in the isolated, perfused guinea-pig heart. The perfusion pressure increase to NPY, which most likely reflects coronary vasoconstriction, was resistant to α- and β-adrenoceptor blockade but sensitive to the calcium antagonist nifedipine. A 50% reduction of the vascular NPY response occurred at 10^{-9} M nifedipine, which did not influence cardiac contractility *per se* or the contractile effects of NA. NPY did not modify the increase in ventricular contractility induced by NA. Noradrenaline did not influence coronary perfusion pressure after β-blockade. Since NPY is present together with NA in cardiac nerves, it may be suggested that NPY is involved in the regulation of NA release as well as the sympathetic control of atrial contractility and coronary blood flow.

Franco-Cereceda, A., *Lundberg, L. M.*, and Dahlof, C.

Acta Physiologica Scandinavica 124:361-369, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, the Astra Foundation; the Swedish Society for Medical Sciences; and the Karolinska Institute.

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MECHANISMS UNDERLYING CHANGES IN THE CONTENTS OF NEUROPEPTIDE Y IN CARDIOVASCULAR NERVES AND ADRENAL GLAND INDUCED BY SYMPATHOLYTIC DRUGS

Neuropeptide Y (NPY) is a recently isolated vasoactive peptide which is present, together with catecholamines, in sympathetic nerves and in the adrenal medulla. In the present study, the authors report that pretreatment with sympatholytic agents influences the tissue levels of NPY-like immunoreactivity (NPY-LI) in the guinea pig. Thus, 24 h after reserpine, noradrenaline (NA) and also NPY-LI were depleted in the heart, spleen and the adrenal gland. The levels of NPY-LI in the vas deferens and stellate ganglia, however, were unaffected by reserpine in spite of marked depletions of NA. The reser-

pine-induced depletion of NPY-LI was probably caused by enhanced nerve-impulse flow and subsequent release from cardiovascular nerves in excess of resupply, since it could be prevented by the ganglionic-blocking agent chlorisondamine. Long-term (6 days) treatment with chlorisondamine reduced the levels of NPY-LI in the stellate ganglion. Short-term treatment (48 h) with guanethidine partially prevented the reserpine-induced depletion of NPY-LI, probably due to inhibition of NPY release. Long-term guanethidine treatment depleted not only NA, but also NPY-LI from the spleen. Pretreatment with the alpha-receptor antagonist phenoxybenzamine did not influence the NA levels but reduced the content of NPY-LI in the spleen via a mechanism that was dependent on intact ganglionic transmission. Since NPY has several cardiovascular actions, changes in NPY mechanisms may contribute to the pharmacological and therapeutical effects of sympatholytic agents.

Lundberg, J. M. et al.

Acta Physiologica Scandinavica 124:603-611, 1985.

Other support: Swedish Medical Research Council, Swedish Tobacco Company, Karolinska Institute, Astra Foundation, and the Austrian Scientific Research Funds.

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NEUROPEPTIDE Y (NPY) REDUCES FIELD STIMULATION-EVOKED RELEASE OF NORADRENALINE AND ENHANCES FORCE OF CONTRACTION IN THE RAT PORTAL VEIN

1. The effect of neuropeptide Y (NPY) on fractional tritium-noradrenaline (^{3}H-NA) release and contractile activity was studied in the isolated portal vein of SHR and WKY rats. 2. NPY (5×10^{-7}M) enhanced the force of the spontaneous contractile activity by about 40%. 3. The fractional ^{3}H-release elicited by transmural nerve stimulation (TNS), which mainly reflects ^{3}H-NA, was reduced by about 40% after preincubation with 5×10^{-7}M NPY in portal veins from both SHR and WKY rats. The inhibitory effect of NPY on TNS-evoked ^{3}H-release was more slowly reversed by washout than the facilitatory action on spontaneous contractile force. 4. The contractile response to field stimulation was not reduced by NPY, but rather tended to be increased. 5. It is concluded that NPY exerts a dual action in the SHR and WKY portal vein, thus enhancing the smooth muscle contractions and inhibiting sympathetic neurotransmission. The inhibitory effect of NPY on TNS-evoked NA efflux, which is present in both SHR and WKY rats, is most likely due to a presynaptic site of action.

Dahlof, C., Dahlof, P., Tatemoto, K., and *Lundberg, J. M.*

Archives of Pharmacology 328:327-330, 1985.

Other support: Swedish Medical Research Council, the Swedish Tobacco Company, Karolinska Institute, and the Astra Foundation.

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